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PROPAGATION OF LABISIA PUMILA VAR. PUMILA (KACIP FATIMAH) USING SEEDS, LEAF CUTTINGS AND TISSUE CULTURE

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ABSTRACT

Labisia pumila is well known as queen of herb in Malaysia due to its phytoestrogenic activity that beneficial to women health. Since the raw material supply is limited, this study is aim to identify feasible propagation methods for future planting stock production of this herb. Three propagation techniques were investigated in this study namely seeds, leaf cuttings and tissue culture. Mother plants of Labisia pumila var. pumila taken from germplasm located in Forest Research Institute Malaysia (FRIM) were used in study. For seeds propagation, a total of 40 seeds were germinated in 100% or river sands and seedlings formed were then transferred to polybags. The growth of the seedlings was recorded for the period of 25 weeks. While for leaf cuttings, matured leaves were used and treated with Seradix 1 (0.1% IBA) before inserted into the rooting medium of the propagation bed. The observation on rooted cuttings was made during 3 to 12 weeks of cuttings. After 12 weeks, the rooted cuttings were transferred into growing medium consisting of top soil: leaf compost: sand (2:3:1) and observed for 25 weeks. For tissue culture technique, combinations of Murashige and Skoog (MS) media with 0.5 mg/L NAA were used for shoot development of L. pumila var. pumila. The established plantlets produced were transferred into greenhouse for acclimatization process for a period of one month. As results, all propagation techniques are able to produce planting stocks of L. pumila var. pumila with more than 80% survival rate. Analysis of variance (ANOVA) revealed that plants produced from tissue culture technique gave the highest leaf number (3.5) and stem height (3.2 cm) compared to other techniques. In conclusion all techniques can be used in multiplication of the species whether for small or large scale production depending on the costs and objectives.

Keywords: Multiplication, growth performance, total phenolic content, production, planting stocks

Introduction

Labisia pumila is an under shrub herb from the Primulaceae family. It is commonly known as kacip fatimah in Malay. At least four varieties of *L. pumila* can be found in Malaysia but only three are regularly reported, namely var. *alata*, var. *pumil*a and var. *lanceolata* (Sunarno, 2005). *Labisia pumila* is synonymous with the title of'queen of herbs' due to its medicinal value for

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women's health. Traditionally, it is used by the local people, particularly Malay women during post-natal care (Jamia, 2006). The development of *L. pumila* as a medical herb has led to its commercialization in the form of health capsules and energy drinks for women, in contrast to its earlier use in water decoction.

As the application of *L. pumila* continuously being explored, lots of new products will be invented, consequently increase the demand of raw material supply. It has been reported that 87% of raw materials used by local herbal industry came from forests and only 17% were cultivated (Rohana et al., 2017). Over the time, our herbal industry will face insufficient supply of local raw materials to feed the growing industry. Therefore, the upstream industries need to ensure a continuous supply of raw materials to meet the anticipated large demand by the local herbal industries Hence, mass propagation of *L. pumila* in commercial scale is relevance to be conducted to avoid such problem. As the demand on this herbal plant keep increasing by the herbal industry, a feasible propagation method of *L. pumila* should be looked into. In this study, three propagation method of *L. pumila* has been identified through seeds, leaf cutting and micro propagation techniques. Some growth parameters such as plant height (cm) and number of leaves produced from the three techniques were evaluated.

MATERIAL AND METHODS

Germination of *L. pumila* var. *pumila* seeds

In this study, seeds were collected from mother plants of *L. pumila* var. *pumila* at the age of two years. The experiment was conducted at the nursery of Forest Research Institute Malaysia (FRIM). The skin and flesh of 40 matured fruits were removed and washed with clean water and treated with fungicide and air-dried for one day. Seeds were then sowed in 100% of river sand in the germination tray and watered every day. The experiment was laid in a Completely Randomized Design (CRD). All method for germination and sowing were similar to the earlier study by Rozihawati (2005). Germination was recorded daily and germinants were counted once cotyledons emerged above the media (Bahuguna et al.,1987). After two months from the germination day, seedlings were harvested and transferred into polybags. Data recorded were percentage of germination, seedling height and number of leaves until the period of 25 weeks.

Propagation of *L. pumila* var. *pumila* by leaf cuttings

A total of 40 leaves were cut into size of 30 cm^2 and rooting hormone (Seradix 1) was applied at the base of the cuttings. Cuttings were grown in an enclosed mist propagation chamber for 12 weeks. Sterile river sand was used as the propagation media. After 12 weeks of treatment with the hormone, the rooted cuttings were transplanted into a growing medium in 8" × 6" polybags.

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The growing media used was a combination of soil, compost and sand in the ratio of 2: 3: 1. The rooted cuttings were transferred to the nursery for growth assessment. The growth of the plants was observed monthly until the week of 25. Among the parameters observed were stem height (cm) and number of leaves.

Propagation of *L. pumila* var. *pumila* by tissue cuture

In vitro leaves of *L. pumila* var. *pumila* obtained from culture initiation stage were used as the explants in this study. Leaves explants with the size of 2 cm^2 were cultured in combinations of Murashige and Skoog (MS) media with 0.5 mg/L NAA. Cultures were incubated at 23 ± 2 ℃ with 16-h photoperiod. The observation on the shoot proliferation was made during 3 to 16 weeks of culture. The established plantlets produced were transferred into greenhouse for acclimatization process for a period of 30 days. A total of 38 plantlets were rooted *ex-vitro* with the application of 0.1% IBA rooting hormone (Seradix 1) and jiffy was used as planting media during the acclimatization process. After a month, all plantlets were shifted to nursery and the growing medium was replaced with top soil: compost: sand at the ratio of 2:3:1. Growth parameters such as stem height (cm) and number of leaves were recorded until the week of 25.

Determination of total phenolic content

Plantlets produced from cuttings and tissue cultures were further tested for analysis of total phenolic content. Seedlings materials were excluded from the analysis since the size of the plants are small and shortage number of samples. A total of 50 grams of fresh leaf samples of *L. pumila* var. *pumila* plantlets were cleaned and washed under running tap water. Fifty gram of the leaf samples were cut into small pieces and soaked in 95 % ethanol (1:3) 300.0 ml for 48 hours. The samples were filtered using Advantec No. 1 paper. The ethanol gathered from the filtration was then evaporated using a rotary evaporator at 46°C. The crude extracts were weighed and stored in a freezer at 4°C for further analysis (Vimala et al., 2003).

Total phenolic content were determined using Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modifications. A 50 mg quantity of plant extracts was extracted for 2 hours with 2.0 ml of 80.0 % methanol containing 1.0 % hydrochloric acid at room temperature on a shaker set at 200 rpm. The mixture was centrifuged at 1000 g for 15 minutes. The supernatant was used for total phenolic assay. The total phenolic determination was by mixing 100.0 µ of supernatant extract with 0.75 ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water). After 5 minutes incubation at room temperature, 0.75 ml sodium bicarbonate (60.0 mg/ml) solution was added and the mixture was allowed to stand at room temperature for 90 minutes. Absorbance was read on a UV-spectrophotometer (Perkin-Elmer

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Lambda 25) at 725 nm. The results were calculated according to the standard curve and expressed as gallic acid equivalents (GAE).

RESULTS AND DISCUSSION

Germination of *Labisia pumila* var. *pumila* seeds

The results showed that seeds of *L. pumila* var. *pumila* germinated uniformly 25 weeks after sowing. Most of *L. pumila* var. *pumila* belonged to polyembryonic group where produced more than one seedlings per seed. From the experiment, it was observed that more than 80% seeds were germinated. Previous study by Hartinie and Azlan (2007) had successfully developed *in-vitro* germination and plantlet establishment of *L. pumila* using seeds as the explants. This species can be propagated through seeds in its natural habitat but the growth rate is very slow. Nevertheless, the seeds are difficult to obtain and probably as one of the reason why very little attempts have been made to cultivate this plant via seeds in herbal industry (Mohd Noh et al., 2002; Syafiqah et al.,, 2015). Freshly collected seeds had the highest viability which declined progressively as the duration of storage increased. To overcome the slow growth performance of the seeds, some pre-treatment on germination of the seeds maybe can be included in future (Anjana and Pramod, 2010). It is important to establish seed germination requirements of *L. pumila* seeds, in order to understand the possible role of different environmental factors for the establishment of the plants in nature.

Propagation of *Labisia pumila* var. *pumila* by leaf cuttings

For leaf cuttings technique, results at 12 weeks after planting in closed misting chamber showed that all 40 leaves of *L. pumila* var. *pumila* cuttings gave percentage of rooting more than 90%. Previous study on propagation of *L. pumila* var. *alata* using cutting technique revealed rooting percentage of 90% and 85%, respectively (Farah Fazwa et al., 2013; Syafiqah Nabilah et al. 2014). Two superior clones of *L. pumila* var. *alata*, namely as LP15 and LP28 also gave high rooting percentages of more than 80% (Nur Nazihah et al., 2016). Similar trend was also reported for rooting percentage of *L. pumila* var. *lanceolata* which gave more than 80% after 9 weeks of propagation. However, study by Rozihawati (2008) showed rooting percentage of *L. pumila* var. *alata* after 12 weeks of propagation was 84% which is higher than *L. pumila* var. *pumila* (72%). In term of plant parts, leaf cuttings gave low percentage (72%) compared to the petiole part (77%) and stem part (83%) (Rozihawati, 2008). As revealed by Norhayati et al. (2013), plant part such as stolon gave 80% in rooting after three weeks of propagation. But presently, propagation by leaf cuttings is preferable as the materials easy to obtain compared to other part such as stem and stolon. Furthermore, production using leaf cuttings also consistently high and available as most researcher focus to use leaf as propagation material.

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Propagation of *Labisia pumila* var. *pumila* by tissue culture

The plantlets establishment of *L. pumila* var. *pumila* through tissue culture was observed more rapid than cutting technique and seed germination. Propagation through tissue culture took about 20 weeks including the acclimatization stage whereas cutting technique took about 24 weeks and seeds germination was 25 weeks. In this study, MS media with combination of 0.5 mg/L NAA was used as the treatment media and gave about 90% survivality after multiplication process. This finding is in line with the study conducted by Syafiqah Nabilah et al., (2016) where *L. pumila* var. *alata* cultured in MS + 0.5 mg/L NAA gave the highest shoot formation compared to other treatment media. The process of plant regeneration in tissue culture is quite similar to leaf cutting where the leaf explants culture will initiate adventitious roots after 2-3 weeks of culture and later after 10 – 12 week the shoots will emerged. According to Hussein and Ibrahim (2014), NAA is the most effective auxin in stimulating rooting compared to IAA. In addition, increasing the concentration of NAA from 1 to 7 mg/L showed increasing value of rooting efficiency which expressed as percentage of rooting, number of roots per explants and dry weight of roots.

Evaluation of growth performances

Based on analysis of variance (ANOVA), *L. pumila* var. *pumila* are able to propagate using seeds, tissue culture and leaf cuttings since the results indicated no significant differences in terms of method of propagation (Table 1). The findings is also supported by Aminah et al. (2008); Rozihawati et al., (2008); Farah Fazwa et al., (2013); Norhayati et al., (2013); Syafiqah Nabilah et al., (2014), which reported that this species are easily produced roots 12 weeks after cutting.

Based on Table 2, in terms of stem height, tissue culture recorded the highest stem height (3.2 cm) and leaf number (3.5) as compared to the other two techniques. Whereas, propagation by leaf cuttings and seeds were not significantly difference in terms of stem height, 2.3 cm and 2.0 cm, respectively. Seeds indicated the smallest (2.6) value of leaf numbers compared to the others.

The addition of plant growth hormones during *in vitro* propagation possibly influenced the vigorous leaf production and stem height of tissue culture plants. This finding is similar with Syafiqah Nabilah et al., (2016), where the application of plant growth hormones on *L. pumila* var. *alata* during *in vitro* regeneration resulted in high production of leaf number (7.80) compared to plant produced from leaf cuttings (4.98).

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Determination of total phenolic contents

Total phenolic contents (TPC) of plant produced through tissue culture and leaf cuttings were compared. The Analysis of Variance (ANOVA) test showed no significant difference between the total phenolic contents of *L. pumila* var. *pumila* from both propagation methods. At six months age, the total phenolic contents in tissue culture plants was about 850 mg/100g/GAE and leaf cuttings plants yield about 650 mg/100g GAE (Table 3).

Syafiqah Nabilah et al. (2016) obtained lower value of TPC for *L. pumila* var. *alata* propagated through tissue culture (217.4 mg/100g GAE) and leaf cuttings (282.5 mg/100g GAE) at the age of six months. It is reported that the value of TPC for variety *pumila* is higher compared to *alata* (Farah Fazwa et al., 2012). However, determination of TPC was only conducted for plants at nursery stages and further evaluation are required to be conducted at the age of 9, 12, 15, 18, 21 and 24 months in order to determine the optimum value of TPC and the best harvesting age of the species. In comparison with Farah Fazwa et al. (2012), screening of total phenolic contents in *L. pumila* var. *alata* collected from five populations are at the range of 971.42±59.53 to 2680.80±25.17 mg/50g GAE. Since the plants were collected from wild, the exact age of the plants is unknown. Age can be one of the factor that influence the difference yield of total phenolic contents in these two study. Achakzai et al. (2009) also reported that aged plant parts usually contained greater level of secondary metabolites.

Different standard used by different investigators also may give different reading of the total phenolic contents. In example, Norhaiza et al. (2009) recorded the values of total phenolic contents in *L. pumila* were between 2.53 to 2.22 mg/g of fresh weight. Whereas Mohd Hafiz et al. (2011) recorded the amount of total phenolic contents in *L. pumila* ranging from 0.23 to 1.01 mg GAE/g dry weight.

CONCLUSION

From this early observation (6 months at nursery stage), it was found that seeds, leaf cuttings and tissue culture are feasible propagation methods for multiplication of *L. pumila* var. *pumila*. Understanding of the dynamics of plant growth and development would help farmers, planters, herbal industries and researchers to get a basic knowledge on this potential herb in the future.

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Table 1. Analysis of variance for comparison of three propagation techniques of *L. pumila* var. *pumila*

Sources	Df	Height (cm)	Leaf number
Methods of production	2	14.91*	7.44*
Error	115		
Total	117		

*significant at 0.05 level of significant.

Table 2. ANOVA for stem height and leaf number between three propagation techniques of *L. pumila* var. *pumila*

Methods of production	N	Stem height (cm)	Leaf number
Cuttings	40	2.34[b]	2.82[ab]
Tissue culture	38	3.20[a]	3.45[a]
Seeds	40	2.02[b]	2.63[b]

Means followed by same letters within each column do not significantly differ at 0.05 level of significance

Table 3: Effects of different propagation methods on the production of total phenolic content

Source of plants	Mean of TPC (mg/100g/GAE) ± SD
Tissue culture	850.0a ± 70.7
Leaf cuttings	650.0a ± 70.7

Means followed by same letters within each column do not significantly differ at 0.05 level of significance